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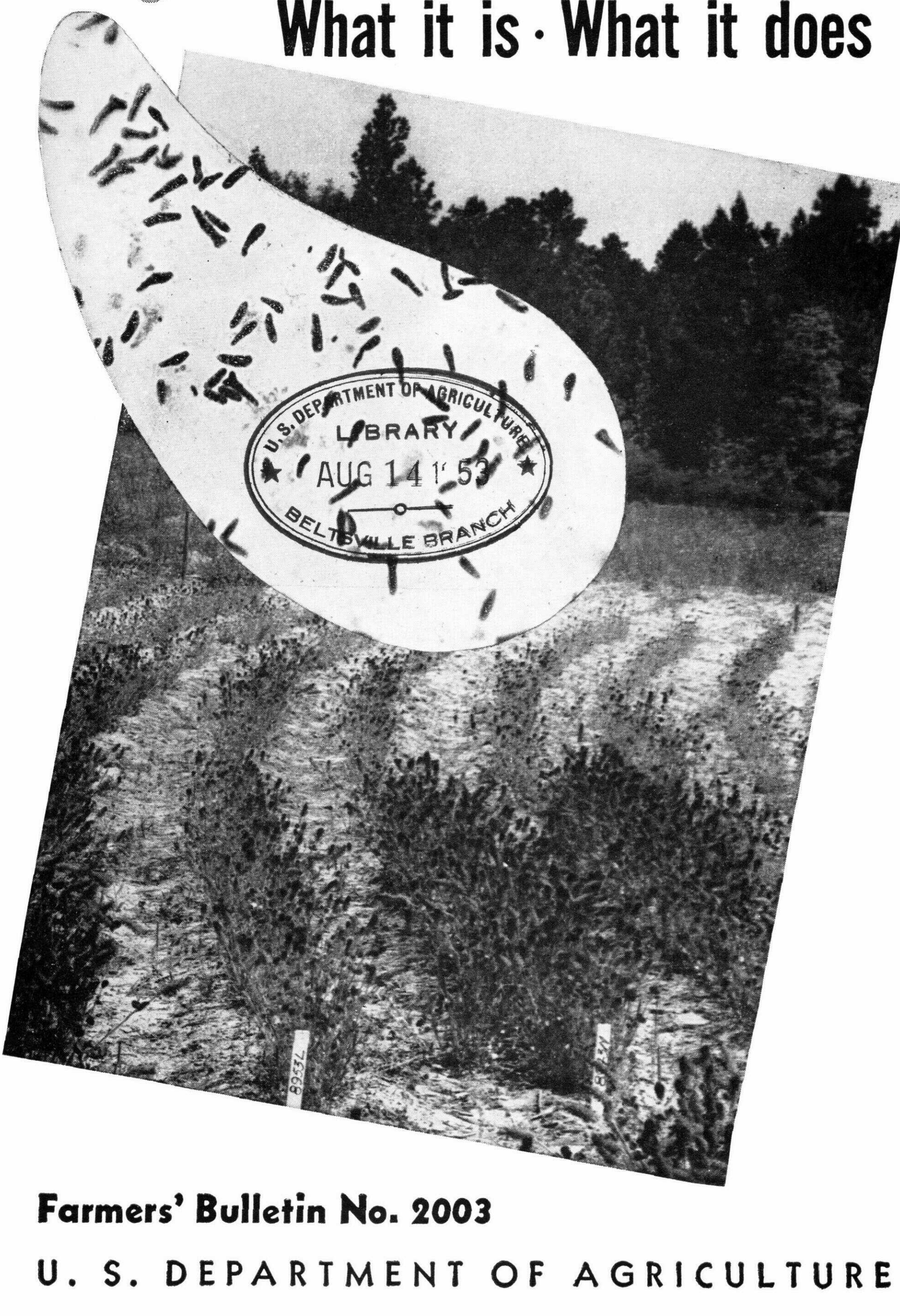
Legume Inoculation

What it is · What it does



Farmers' Bulletin No. 2003

U. S. DEPARTMENT OF AGRICULTURE

For Successful Legume Inoculation—

Use the right inoculant for the legume.
Keep commercial culture in cool, dark place until used.
Follow directions and mix culture well with seed.
Plant seed within 48 hours after inoculated, or reinoculate.
Inoculate in all cases of doubt and always on new land.
Prepare a good, well-fertilized, moist seedbed; after planting small seeds, cultipack the soil.

COVER-PAGE ILLUSTRATION *shows the effectiveness of different cultures or strains of clover bacteria on crimson clover.*

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LEGUME INOCULATION: WHAT IT IS; WHAT IT DOES

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Contents

LEGUMES are plants that bear their seeds in pods, like a bean. They are used for hay, silage, seed, winter cover crops, or for pasture.

Inoculation of legumes means the introduction of legume bacteria into the soil for the purpose of increasing the production of successful legume crops, which also improve the soil.

The actual increase in legume-crop growth resulting from inoculation depends upon the legume bacteria used and also upon soil and climatic conditions.

Well-inoculated legumes have nodules (small lumps) on the roots, produced by effective legume bacteria. All three—the legume, the nodule, and the bacteria—are necessary for the nitrogen-fixation process. The nitrogen fixed may vary from 50 to 100 pounds or more per acre per year, depending on the plant, the soil, and the climatic conditions.

Inoculation with effective bacteria may mean the difference between success and failure of the legume crop. The relative importance of leguminous plants for food, for livestock feed, for soil improvement, for soil conservation, and for industrial products depends primarily upon the effectiveness of the nitrogen-fixing bacteria living in the nodules on the legume plant roots.

Legume Inoculation, an Important Discovery

The beneficial association of soil bacteria with legumes was discovered in 1886. This discovery proved beyond doubt that legume bacteria are essential to legumes. The bacteria enables the legumes to use the nitrogen from the air, building it into protein, and to enrich the soil in which they grow. The discovery had such practical results that, almost immediately, many laboratory and field studies were begun to learn more about the activities of these micro-organisms, now known as legume bacteria.

In 1901 the United States Department of Agriculture began its investigations on methods of inoculating legumes. Since then the Department has given farmers accurate information on how to increase crop yields by this practice and also has pointed out to scientific workers the precautions they must take to get satisfactory results. Many State agricultural experiment stations also have issued bulletins, circulars, and scientific papers, and several have published books on the subject of legume bacteria.

As our knowledge of legume-bacteria relationships increases we encounter increasing numbers of legume-inoculation problems. Changing soil and climatic conditions, various cropping sequences, methods of land preparation, different soil treatments, and varied uses of the crop affect the legume-bacteria relationship. The successful introduction of a new species or variety of legume often depends on the effective strain of legume bacteria being present when the seeds are planted.

The Legumes

Of the more than 10,000 known species of legumes, only about 200 are cultivated by man, and in the United States only about 50 are grown commercially. These species are further divided into varieties. For instance, more than 100 named varieties of soybeans are being grown in this country.

The legumes are rich in high-quality protein, are well supplied with phosphorus and calcium, and are a good source of vitamins, especially A and D. These qualities make legumes rate as one of man's best foods and as almost indispensable for efficient, economical livestock feeding.

The protein in legumes is directly related to high nitrogen content, and in this respect they differ markedly from grasses and other non-legumes. In one instance, the average protein content of 1 ton of each of eight legume hays was compared with the protein in eight grasses. The legumes averaged 304 pounds of protein per ton and the grasses 156 pounds. The protein content of legumes will vary with the stage of maturity. For example, before bloom, alfalfa has about 380 pounds of protein per ton and red clover about 375 pounds, as compared with the averages at harvest of 295 and 235 pounds, respectively.

The following data, obtained at the Plant Industry Station, at Beltsville, Md., in 1949, show how the stage of maturity influences the percentage of crude protein in Ladino clover:

Date and stage of maturity:	*Percent*
May 6, no flower heads	30
May 26, flower heads showing	27
June 13, past full bloom	20

Legumes have gained great popularity and economic importance wherever they have been grown. This is because (1) they have high nitrogen content and feeding value, (2) they contain a large proportion of readily decomposable organic matter, and (3) they are versatile in fitting into special farm practices.

Nitrogen and Root Nodules

The air we breathe is primarily a mixture of nitrogen and oxygen gases. About 80 percent by volume is pure nitrogen in a free or un-

combined state. Above every acre of land surface there is about 35,000 tons of this free nitrogen, which, as such, is totally useless to plant or animal life. Nitrogen has been called the aristocrat of all the elements—it is stubbornly opposed to entering into combination with other elements. However, under powerful influences—such as lightning discharges and chemical reactions brought about by tremendous heat—the air nitrogen does combine to form compounds that are used to supply industrial and agricultural needs.

Fortunately, for obtaining atmospheric nitrogen for farming, nature provides farmers with a simpler and less expensive method—the growing of inoculated legumes. Soon after the legumes begin to grow, the legume bacteria invade the tiny root hairs and multiply in large numbers, forming growths called nodules. A definite partnership is established—one representing a true symbiosis, or a living together of two organisms to the advantage of both—the legume plant furnishes the necessary sugar or energy, and the bacteria uses this energy material to fix the free nitrogen of the atmosphere and gives it directly to the plant. This is called nitrogen fixation.

Just how these bacteria do this work is still unknown. Apparently, however, they work with remarkable ease, because experiments have given evidence of nitrogen fixation after 2 or 3 weeks. A deeper, darker green in inoculated legumes than in those uninoculated is one sure sign of nitrogen fixation by the bacteria. In fact, color differences are more reliable for judging nitrogen fixation than numbers of nodules.

Inoculated legumes growing in normal soils display definite characteristic types of nodule formation. Some of these types are shown in figures 1, 2, and 3.

The clustering of nodules around the taproot at the point where the inoculated seed is planted generally indicates that the nodules were formed by the bacteria added in the inoculant. If the inside of the nodule is red, this indicates high nitrogen-fixing activity. Nodules scattered over the side roots are usually formed by the legume bacteria naturally present in the soil.

In examining legumes to note the effects of seed inoculation it is always well to dig plants at different stages of growth. Nodules come and go with varying moisture levels in the soil. Three examinations should ordinarily be made—the first about 3 or 4 weeks after planting, the second during the middle of the growing period (if moisture conditions are favorable), and the third when the legume is in full blossom. As the legumes mature, the nitrogen compounds formed in the nodules furnish nitrogen for the building of proteins in the leaves, stems, and seed. In the early stages the nodules may contain 5 to 8 percent nitrogen, but at seed maturity they are no richer in nitrogen than the rest of the root. The nodules disintegrate rapidly at the time of seed formation.

Nitrogen Fixation by Legumes

The quantity of nitrogen taken from the air and fixed by the legume bacteria for different legumes is difficult to calculate. It varies with (1) the kind of legume, (2) the effectiveness of the legume bacteria, (3) the soil conditions, and (4) the presence of necessary plant-food elements exclusive of nitrogen. In high-fertility soils well supplied with available nitrate nitrogen there may be little or no fixation, as

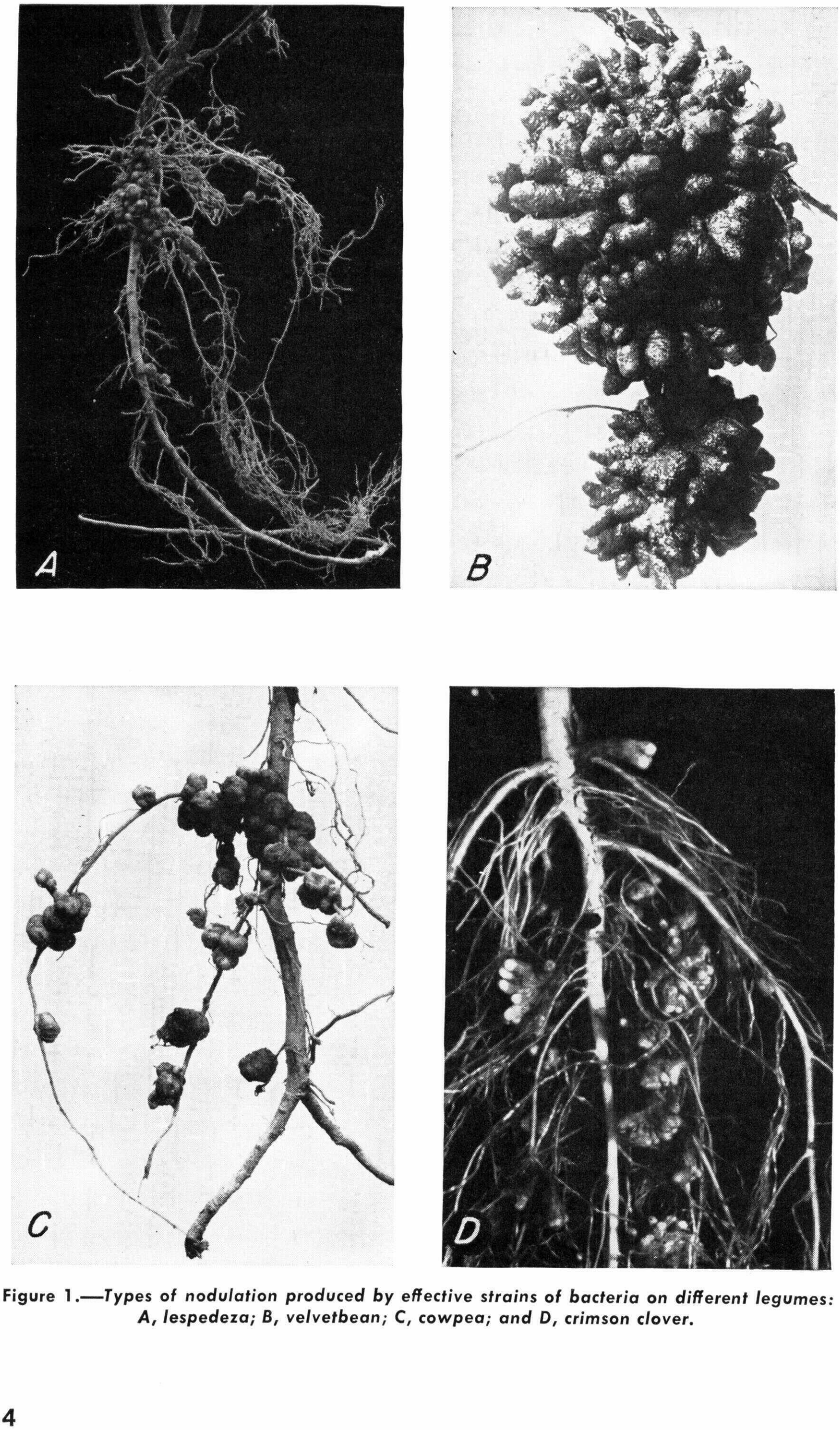

Figure 1.—*Types of nodulation produced by effective strains of bacteria on different legumes: A, lespedeza; B, velvetbean; C, cowpea; and D, crimson clover.*

the legume plants seem to use this available nitrogen rather than encourage the bacteria to fix more of it. Most noticeable results from legume inoculation are obtained on soils of average fertility or on rundown depleted soils.

Those factors, exclusive of nitrogen, that make for the optimum growth of legumes play an important part in increasing the quantity

Figure 2.—*Highly effective nodulation on soybean—clusters around taproot produced by inoculant added to seed; nodules on side roots formed by soybean bacteria in the soil.*

of nitrogen that is fixed by legumes. Usually a combination of growth factors reacting favorably governs how much nitrogen is fixed. Although it is not possible to answer with directness the question of how much nitrogen is fixed by the bacteria in the nodules on legume plant roots, different investigators have reported their findings on the most important legumes (table 1). Alfalfa, the clovers, and lupines lead other legume crops in the amount of nitrogen fixed per acre; peanuts, beans, winter peas, and garbanzo fix much lower quantities of nitrogen.

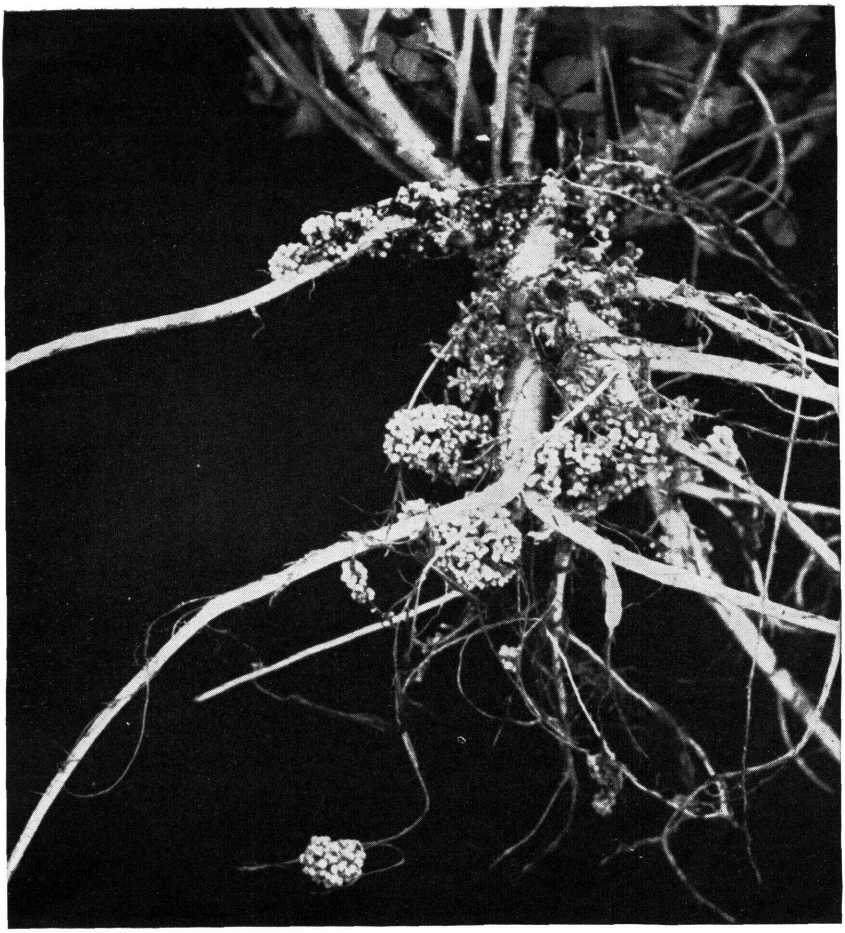

Figure 3.—*A sweetclover root, showing excellent nodulation.*

TABLE 1.—*Estimated amount of nitrogen fixed from the air by legume bacteria in different legumes grown in the United States*

Legume	Average amount nitrogen fixed per acre	Legume	Average amount nitrogen fixed per acre
	Pounds		*Pounds*
Alfalfa	194	Vetch	80
Sweetclover	119	Beans	40
Red clover	114	Peanuts	42
Ladino clover	179	Lupines	151
White clover	103	Velvetbeans	67
Alsike clover	119	Sourclover	98
Crimson clover	94	Kudzu	107
Lespedezas (annual)	85	Fenugreek	82
Soybeans	58	Bur-clover	78
Peas	72	Lentils	103
Cowpeas	90	Garbanzo	66
Winter peas	50	Pastures with legumes	106

The Legume Bacteria

Legume bacteria are single-celled micro-organisms that vary in size and shape with age and with the composition of the medium in which they grow. Under the ordinary microscope with a magnification of 1,000 diameters they may be either the usual rod forms, 0.5 to 0.9 micron wide and 1.2 to 3 microns long (a micron is 1/25,000 inch), or the irregular X or Y, or club-shaped, forms shown in figure 4.

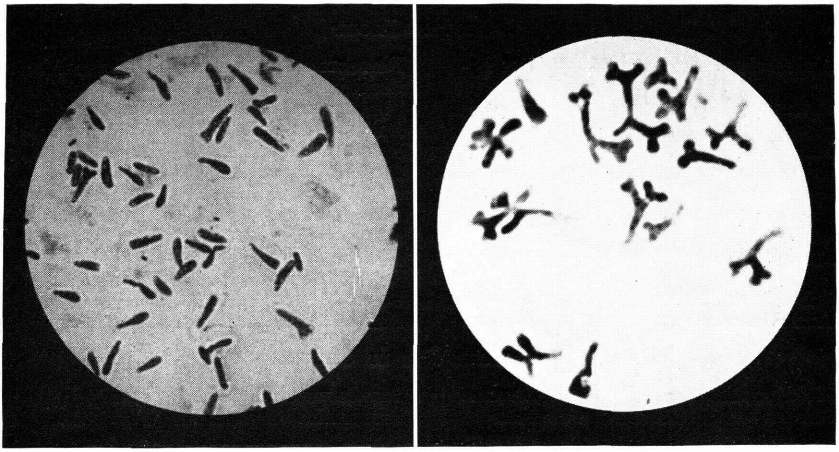

Figure 4.—Appearance of legume bacteria through a powerful microscope: Left, Clover bacteria; right, irregular X and Y shapes.

When young, legume bacteria are extremely active; they have been observed to have either one polar flagellum (propeller organ) or a number of flagella surrounding the cell.

That legume bacteria are of different kinds has been known for a long time. For example, the bacteria that work on alfalfa and sweetclover will not function on the clovers or on peas, beans, soybeans, and other legumes. Conversely, the clover organisms fail to work on alfalfa and sweetclover.

The fact that the legume bacteria are so definitely selective was responsible for the recognition of so-called cross-inoculation groups of leguminous plants. Any plant within such a group can be inoculated with a culture of the right kind of bacteria, usually comprising several strains known to inoculate effectively all the legumes in that particular group

Farmers have been accustomed to ordering legume cultures according to group designations, such as the alfalfa group, the clover group, the pea and vetch group, and others. Seven of these groups are now recognized. They are given below, with a list of the most important legumes in each.

Alfalfa Group

Common name	Scientific name	Common name	Scientific name
Alfalfa	*Medicago sativa*	Tifton bur-clover	*M. rigidula*
Buttonclover	*M. orbicularis*	Yellow alfalfa	*M. falcata*
California bur-clover	*M. denticulata*	White sweetclover	*Melilotus alba*
Spotted bur-clover	*M. arabica*	Hubam sweetclover	*M. alba annua*
Black medic	*M. lupulina*	Yellow sweetclover	*M. officinalis*
Snail bur-clover	*M. scutellata*	Bitterclover (sourclover)	*M. indica*
Tubercle bur-clover	*M. tuberculata*	Fenugreek	*Trigonella foenumgraceum*
Little bur-clover	*M. minima*		

Clover Group

Alsike clover	*Trifolium hybridum*	Berseem clover	*T. alexandrinum*
Crimson clover	*T. incarnatum*	Cluster clover	*T. glomeratum*
Hop clover	*T. agrarium*	Zigzag clover	*T. medium*
Small hop clover	*T. dubium*	Ball clover	*T. nigrescens*
Large hop clover	*T. procumbens*	Persian clover	*T. resupinatum*
Rabbitfoot clover	*T. arvense*	Carolina clover	*T. carolinianum*
Red clover	*T. pratense*	Rose clover	*T. hirtum*
White clover	*T. repens*	Buffalo clover	*T. reflexum*
Ladino clover	*T. repens* (*giganteum*)	Hungarian clover	*T. pannonicum*
Sub clover	*T. subterraneum*	Seaside clover	*T. wormskjoldii*
Strawberry clover	*T. fragiferum*	Lappa clover	*T. lappaceum*
		Bigflower clover	*T. michelianum*
		Puff clover	*T. fucatum*

Pea and Vetch Group

Field pea	*Pisum arvense*	Purple vetch	*V. atropurpurea*
Garden pea	*P. sativum*	Monantha vetch	*V. articulata*
Austrian Winter pea	*P. sativum* (var. *arvense*)	Sweet pea	*Lathyrus odoratus*
Common vetch	*Vicia sativa*	Rough pea	*L. hirsutus*
Hairy vetch	*V. villosa*	Tangier pea	*L. tingitanus*
Horsebean	*V. faba*	Flat pea	*L. sylvestris*
Narrowleaf vetch	*V. angustifolia*	Lentil	*Lens culinaris* (*esculenta*)

Cowpea Group

Cowpea	*Vigna sinensis*	Guar	*Cyamopsis tetragonoloba*
Asparagus-bean	*V. sesquipedalis*	Jackbean	*Canavalia ensiformis*
Common lespedeza	*Lespedeza striata*	Peanut	*Arachis hypogaea*
Korean lespedeza	*L. stipulacea*	Velvetbean	*Stizolobium deeringianum*
Sericea lespedeza	*L. cuneata*	Lima bean	*Phaseolus lunatus* (*macrocarpus*)
Slender bushclover	*L. virginica*	Adzuki bean	*P. angularis*
Striped crotalaria	*Crotalaria mucronata*	Mat bean	*P. aconitifolius*
Sunn crotalaria	*C. juncea*	Mung bean	*P. aureus*
Winged crotalaria	*C. sagittalis*	Tepary bean	*P. acutifolius* var. *latifolius*
Florida beggarweed	*Desmodium tortuosum*	Partridge-pea	*Chamaecrista fasciculata*
Tick trefoil	*D. illinoense*	Acacia	*Acacia linifolia*
Hoary tickclover	*D. canescens*	Kangaroo-thorn	*A. armata*
Kudzu	*Pueraria thunbergiana*	Wild-indigo	*Baptisia tinctoria*
Alyceclover	*Alysicarpus vaginalis*	Hairy indigo	*Indigofera hirsuta*
(No common name)	*Erythrina indica*		
Pigeonpea	*Cajanus cajan* (*indicus*)		

Bean Group

Garden beans, kidney bean, Navy bean, pinto bean	*Phaseolus vulgaris*	Scarlet Runner bean	*P. coccineus* (*multiflorus*)

LUPINE GROUP

Common name	Scientific name	Common name	Scientific name
Blue lupine	*Lupinus angustifolius*	Sundial	*L. perennis*
Yellow lupine	*L. luteus*	Texas bluebonnet	*L. subcarnosus*
White lupine	*L. albus*	Serradella	*Ornithopus sativus*
Washington lupine	*L. polyphyllus*		

SOYBEAN GROUP

All varieties of soybeans	*Glycine max* (*Soja max*)

This grouping of legumes is of great practical value, for it is obviously not necessary to have a specific culture of legume bacteria for every legume to be planted. ***It is necessary and extremely important, however, to have a sufficient number of different strains of known effectiveness in an inoculant to inoculate all the legumes specified on the culture label.***

The following legumes appear to require specific strains of legume bacteria for effective inoculation:

SPECIFIC STRAIN GROUP

Common name	Scientific name	Common name	Scientific name
Birdsfoot trefoil	*Lotus corniculatus*	Sanfoin	*Onobrychis vulgaris* (*sativus*)
Big trefoil	*L. uliginosus*	Crown vetch	*Coronilla varia*
Foxtail dalea	*Dalea alopecuroides*	Siberian pea-shrub	*Caragana arborescens*
Black locust	*Robinia pseudoacacia*	Garbanzo	*Cicer arietinum*
Trailing wild bean	*Strophostyles helvola*	Leadplant	*Amorpha canescens*
Hemp sesbania	*Sesbania exaltata*		

It has been known for a long time that nodules do not form on some legumes. Among these are redbud (*Cercis canadensis*), Kentucky coffeetree (*Gymnocladus dioicus*), honeylocust (*Gleditsia triacanthos*), and sicklepod (*Cassia tora*). Nodules have been found on another legume, Kura clover (*Trifolium ambiguum*), but so far an effective strain of legume bacteria for it has not been isolated.

Strain Variation Among Legume Bacteria

That not all legume bacteria are the same has been repeatedly emphasized. Some prefer certain specific groups of legumes, others only a single species. Some varieties, particularly soybeans and peas, have specific legume bacteria preferences. A strain may produce excellent results on one variety, but it would be a poor nitrogen fixer on another variety.

Another difference is the variation in effectiveness between strains of bacteria isolated from the same legumes and from different legumes within the same group. This type of strain variation among the legume bacteria has great practical significance. (See p. 11.) As nitrogen fixers, some are high, some are poor, and others show gradations between these extremes.

The method for studying strain variation has also shown the existence of parasitic strains of legume bacteria. These parasitic, or ineffective, strains enter the root and form numerous small nodules, but fail to fix any nitrogen or otherwise benefit the plant. The nodulation of guar plant roots produced by one ineffective strain and by two effective strains is shown in figure 5.

This discovery of parasitic strains has made the number of nodules of less importance as a measure of value of a legume inoculant. Numbers of nodules fail to tell the whole story of the effectiveness of the bacteria in fixing nitrogen. It is necessary to measure plant-growth responses, particularly mass, vigor, color, and, if possible, total nitrogen content. Producers of commercial cultures today realize the great importance of using only highly effective nitrogen-fixing strains of bacteria in the preparation of their legume inoculants. Many bacteria of effective strains added to legume seeds will prevent the entrance of ineffective strains already in the soil. The search for new and better strains is continuous, for the cultures that prove of greatest benefit under field conditions will be the ones in greatest demand by

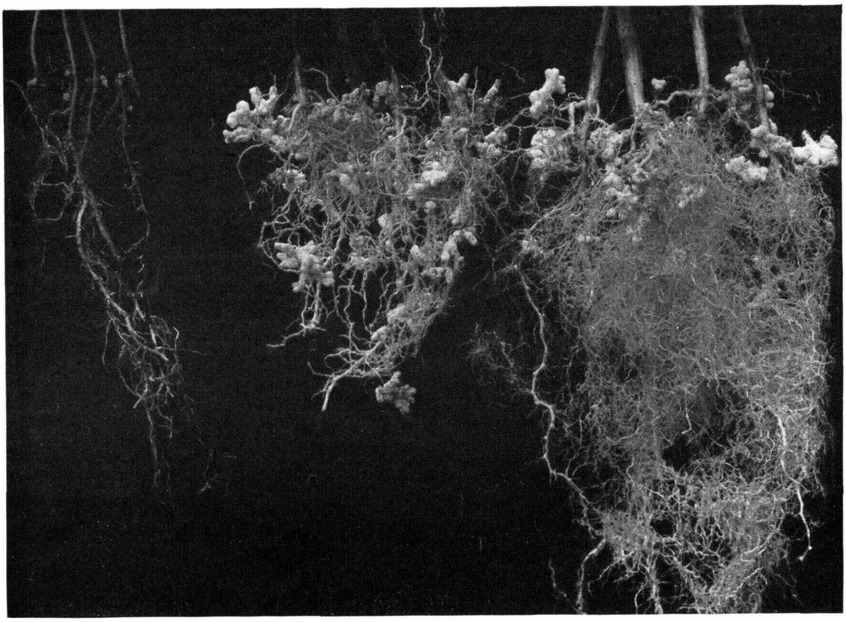

Figure 5.—*Guar plants, showing (left) small parasitic types of nodules and (right) nodulation of two effective bacteria strains. All plants were grown under the same conditions.*

farmers. Strain variation among legume bacteria is illustrated in figures 6 to 9.

Some important observations should be emphasized.

1. The alfalfa and sweetclover strains will work on alfalfa or sweetclover equally well, but they fail to produce nitrogen fixation in bur-clovers and fenugreek. Strains from bur-clover and fenugreek, on the other hand, will work and fix nitrogen on bur-clovers, fenugreek, alfalfa, and sweetclovers.

2. Strains from red and white clovers fix nitrogen on their host plants, but not all of them will effectively inoculate crimson clover. One strain isolated from berseem clover was effective on all clovers tested except the white and the red.

3. Strains of legume bacteria show definite varietal preferences. For example, some soybean bacteria work on one or two soybean varieties better than on others. The same is true for different varieties of canning or freezing peas.

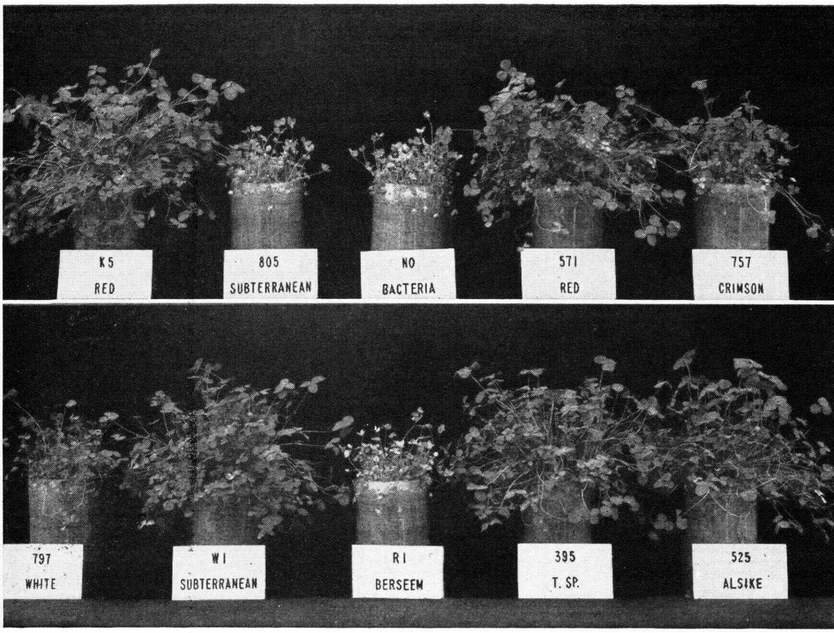

Figure 6.—*Effect of different strains of clover bacteria on Ladino clover.*

Figure 7.—*Effect of four strains of alfalfa-sweetclover bacteria on alfalfa. The culture used in the third pot from the left was definitely parasitic.*

4. Strains of legume bacteria from birdsfoot trefoil may be highly effective on its host but totally ineffective on big trefoil, which is another species. Strains that may be highly effective on big trefoil

Figure 8.—*Effect of four strains of clover bacteria on crimson clover. The poor strain used in the container on the left was effective on both red and white clover.*

fail to work on birdsfoot trefoil. However, experimental work has shown that when effective strains for each of these trefoils are mixed together into a single inoculant, both species can be satisfactorily inoculated with such a culture.

Figure 9.—*Effect of five strains of legume bacteria on bur-clover. The bacteria used were isolated from (A) black medic, (B) bur-clover, (C) alfalfa, (D) fenugreek, and (E) sweet-clover.*

Greenhouse tests of different strains of legume bacteria enable the bacteriologist to select the best strains for a given legume. Field tests of these selected strains are then desirable to see how they affect the legumes when they are grown on the farm (fig. 10).

Figure 10.—*Effect of different strains of clover bacteria on white clover. Several strains were no better than the uninoculated row shown in the right-hand corner.*

Necessity for Inoculation

Farmers cannot be sure before they plant a legume that sufficient bacteria of the proper kind are present in their soils. Nor can they be sure that the bacteria in the nodules on a previous legume were of maximum benefit to that crop. It is entirely possible that the bacteria left in the soil may lose their beneficial properties, that is, their ability to fix appreciable quantities of air nitrogen.

Too often it is taken for granted that inoculation is not necessary, because a legume has been grown in the same soil. If the proper bacteria are not present the young legume plants look spindly, sickly, or yellow and may or may not show nodules on the roots. Such cases present a real problem, because it is so much more difficult to get growing plants inoculated than to inoculate the seeds before planting.

The one fundamental purpose of legume inoculation is to add a fresh culture of effective strains of legume bacteria to the seed (preferably), so that when the young plant begins to grow, the bacteria will be right there to enter the tiny root hairs and begin their beneficial work in the early stages of the plant's growth.

Now that farmers can buy legume inoculants prepared with the most effective strains, the simplest, easiest, and most economical way to insure successful growth is to inoculate legume seeds before each planting. Good sound advice to all farmers is to inoculate all winter annual legumes in the South when they are planted, and to inoculate

legume seeds in other areas when they are planted for the first time and especially on new land.

Conditions That Affect Legume Bacteria

In the natural competition between inhabitants of the soil, the legume bacteria have the advantage that they are protected from time to time in nodules. Without this association, they would have adverse soil conditions to cope with. Conditions essential to the satisfactory growth of legumes must be fulfilled before maximum results with inoculation can be expected. The principal requirements are the proper preparation of the soil; the presence of an available supply of lime, phosphorus, and potash; and the use of healthy adapted viable seed.

Once legume bacteria are in the soil, they are subject to the conditions existing there; if the chemical reaction of the soil is suitable, if sufficient moisture and plant food is available, and if temperatures are not too high, the bacteria should function normally. Acid soils tend to eliminate the bacteria to the degree that these organisms are not tolerant of acid conditions. Bacteria of alfalfa, sweetclover, and red clover are among those that are not very acid-tolerant. Soybean, velvetbean, cowpea, vetch, lespedeza, and lupine bacteria belong to the acid-tolerant types.

Cultures of legume bacteria are alive and should be treated as living things. They will tolerate low temperatures much better than high. Exposing them to heat that is unbearable by man, or even uncomfortable, may impair or destroy their effectiveness. Inoculating material should therefore be stored in a cool place until used.

Although legume bacteria will tolerate sunlight to some degree, avoid unnecessary exposures either of the unopened containers or of seed that has been treated. Bacteria that dry on seed soon die. If inoculated seed must be kept as long as 48 hours it is advisable to reinoculate. For this reason the purchase of preinoculated seed is not advisable.

Seed treated with legume bacteria should not come in direct contact with caustic lime or mixed fertilizers. Inoculated seed may be drilled down the same spout with superphosphate or basic slag without injury to the bacteria. If the concentration of fertilizer does not injure seed germination it will not ordinarily harm legume bacteria.

Most seed disinfectants are toxic to legume bacteria. Consequently, legume seed that has been treated with disinfectant compounds should not be inoculated in the usual manner. In large-scale operations, the inoculum is mixed with wheat middlings, sawdust, or other inert material and drilled in advance of planting. This practice, called "preplanting" of the bacteria, is successful in large pea-growing areas where it becomes necessary to treat the seed.

Winter Annual Legumes in the South

Although an intensive winter annual-legumes cover-crop program in the South has been in operation for some time, agricultural authorities agree that it is not always easy to obtain effective inoculation. The crops giving the most trouble are crimson clover, lupines, winter peas, and vetches.

When winter annual legumes are grown in rotation and follow cotton, corn, or sorghum, the inoculated legume seeds are usually broadcast on the soil in the early fall. If a cultipacker is used and if rains follow soon after seeding, the legume bacteria get into the soil, and under favorable conditions good inoculation is obtained. If, on the other hand, conditions are unfavorable and a prolonged dry spell with excessive high temperatures follow the seeding, many of the bacteria may be killed and poor inoculation follows. If this happens, it is advisable to use supplemental inoculation as suggested on page 17.

When winter annual legumes are seeded in pastures it is essential to inoculate with effective legume inoculants. The grass sod furnishes protection for the legume bacteria, and, when fertility requirements have been met, excellent inoculation results usually follow. After good inoculation of legumes grown with perennial grass is once established, it is not necessary to reinoculate, except in cases where the legume fails to grow normally.

Bermuda grass sod furnishes protection and provides a favorable growth medium for the bacteria of crimson clover, as shown in figure 11. This was the first year crimson clover was seeded on this land. All seeds were inoculated with the same culture. The time and rate of seeding was the same, the dark areas were on sod, the light areas were

Figure 11.—*The dark heavy growth of crimson clover was obtained when inoculated seed was sown in Bermuda grass growing in rows in a nursery. The crop failed between the rows where no sod was present.*

clean-cultivated. This is a striking example of the value of sod protection in establishing successful inoculation in Southern States.

Even when successful inoculation is obtained with the first seeding of a legume, it is not safe to assume that sufficient numbers of legume bacteria will be in the soil the next time the same legume is planted. Bacteria are off legume plants while a row crop or intertilled crop is grown, and live free in the soil during the summer when unfavorable environmental conditions are present. The frequent stirring or cultivation of the soil exposes the bacteria to drying, sun's rays, and high temperatures, and many of the organisms are killed. The number surviving may be insufficient to produce maximum inoculation on the next legume crop.

Preparation and Use of Commercial Cultures

Soon after the discovery that legume bacteria can fix nitrogen in nodules on the plant roots, soil bacteriologists began to cultivate these organisms on artificial mediums in the laboratory. A few years later, prepared cultures were offered for sale to farmers. These early cultures were made with specific bacteria for a specific kind of legume, and under field conditions their use resulted in varying degrees of success.

The few failures did not discourage the originators of commercial cultures. Greater success came as they developed more suitable mediums and improved methods of application. Gradually a farmer demand was created for commercial inoculants, and a number of companies began to produce legume cultures and to sell them mostly through seed dealers.

To prepare effective cultures for legumes, the persons engaged in the work must have specialized training and experience. Adequate laboratory facilities, equipment for controlled production, and greenhouse space or other suitable means for testing plants are prerequisites to satisfactory production.

Routine work in such a laboratory calls for periodic tests and transfers of all strains of legume bacteria used in the production of commercial inoculants. These tests are made for purity and for effectiveness on the growing plants. New strains are isolated each year, and this is an important feature of the work. These must be purified and tested also. A selection is made of the most effective strains, and a given number are used for the production of the different culture groups. For example, 5 or 6 strains may be used for a culture to inoculate the alfalfa group, whereas 10 to 12 strains may be used in the production of a soybean inoculant.

The bacteria are grown either in liquids or on the surface of agar. Heavy suspensions of bacteria containing an excess supply of food are used for mixing with the carrier, which may be a finely ground peat, or mixtures of peat and charcoal, peat and sand, or other materials. The three types of carriers generally used are: (1) moist humus or finely ground peat, (2) agar, and (3) liquid. The bulk of commercial inoculants are prepared in moist humus. Most seed suppliers handle one or more brands of commercial legume inoculants and thus make them readily available to the consumer trade. *Because of the perishable nature of these living bacteria, dealers are cautioned not to store the cultures in places that are either too warm or too dry.*

Be sure that the culture is prepared for the specific seed you wish to plant, and use it before the expiration date.

In using agar cultures, add a small quantity of clean cool water to the bottle, shake it vigorously to get the bacteria in suspension, add more water, and then pour the bacterial suspension on the legume seeds and mix them until all are moistened.

In using peat or humus-type inoculants, either (1) moisten the seeds with water and empty the contents of the container on them, mixing until all are coated with the black substance; or (2) add a specified quantity of water to the inoculant to form a thin paste and pour it on the seeds.

Specific directions for using commercial cultures are found on the label of each container.

A thorough mixing is essential in all cases; do not use too much water; avoid soaking the seeds.

Plant the seeds as soon as possible after they are inoculated with legume-bacteria cultures. Ideal conditions prevail shortly before a gentle rain. When inoculated small legume seeds have remained on or near the surface of the soil, exposed to hot drying winds for several weeks, supplemental inoculation is advisable. This may be done by mixing a legume inoculant with cottonseed meal, wheat middlings, or even sand and broadcasting the mixture over the soil immediately before or after a rain.

When young legume plants show lack of proper inoculation it may be desirable to reseed the area with inoculated seeds. The bacteria added on the seed may eventually gain access to the root hairs of the growing plants and produce successful inoculation.

When soybeans are planted in a soil for the first time, marked color differences and growth effects may be noted between inoculated and uninoculated or inefficiently inoculated plants (fig. 12).

Figure 12.—*A field test of different soybean cultures. At the left the dark-green growth was brought about by effective nitrogen-fixing soybean bacteria. In the right foreground soybeans inoculated with several unsuited cultures could not be distinguished from the uninoculated yellowish-green plants.*

The demand for effective legume inoculants has shown a rapid and steady increase since World War I. Probably no commodity purchased by farmers gives greater returns per dollar invested. The average cost for soybean inoculants when purchased in units of large size is about 10 cents per each bushel; the cost of the 1-bushel-

size unit for alfalfas and clovers is about 50 cents. This size will inoculate 3 to 5 acres, depending upon the rate of seeding. Seed inoculation is cheap crop insurance for legumes.

Inspection of Legume Inoculants

To protect farmers from buying worthless cultures, certain control agencies have been set up. In 1916 the United States Department of Agriculture began testing commercial legume inoculants in accordance with an act of Congress providing for soil microbiological investigations. Cultures were procured each year in the open market and tested on the legume plants for which they were recommended. From time to time results of these tests were published along with the names of the manufacturers or distributors. This testing work is still carried on by the Department. The testing process consists of inoculating seed according to directions on the container and planting them in sterile sand moistened with a sterile nitrogen-free nutrient solution. Great care is taken to prevent entry of legume bacteria and the transfer of bacteria from one seed pot to another. A culture is considered satisfactory if under these conditions it produces nodules, increases plant growth, and produces a plant that is darker green than uninoculated controls. Field tests are sometimes made (figs. 13 and 14).

In addition to testing commercial legume inoculants, the Department has kept in close touch with inoculant producers. Its representatives have visited the more important laboratories and offered to help the manufacturers in production problems and thus assure better cultures for the farmer. A few of the States also have control agencies to protect their farmers from unscrupulous producers and dealers. All these agencies have had a decidedly beneficial effect in bringing

Figure 13.—*Austrian Winter peas respond to inoculation. Two good cultures were used on the rows to the left, and the typical nodulation produced is shown in the inset. Where no inoculation was used (on right) the plants failed to make satisfactory growth.*

Figure 14.—*Effect of proper inoculation of hairy vetch, one of the best winter cover crops for certain sections of the South. The uninoculated row on the right was almost a failure. Typical vetch nodulation is shown in the inset.*

about improvements and raising the standard and quality of inoculants.

Commercial cultures for legumes have reached a high state of reliability and usually produce satisfactory results.

Economic Importance of Legume Inoculation

Effective inoculation of legumes has been a major factor in improving their yield and quality. Legume inoculation makes available greater quantities of the high-protein feeds so necessary in livestock production. Legumes add much nitrogen to the soil and in addition their growth contributes greatly to the maintenance of good-quality organic matter. Organic matter improves the physical property of the soil, increases its moisture-holding capacity, and holds plant nutrients. The combination of organic matter and necessary fertilizers provides a readily available supply of plant nutrients for crop production.

Inoculated legumes, as indicated above, play three important roles in the world's food program: (1) They increase the supply of high-protein feeds for domestic animals; (2) they increase the yield of many legumes valuable to man for food; and (3) indirectly, they increase the production of other farm crops.

Not all agricultural soils contain the bacteria necessary to promote successful growth in legumes. Furthermore, many of the legume bacteria naturally present in cultivated soils are not high nitrogen-fixing strains. For example, 100 soybean fields in Wisconsin were examined and nodules collected for isolating the soybean bacteria.

When these strains were tested on soybeans it was found that 25 percent were highly effective, 50 percent only average, and the rest poor or ineffective. In Kansas, 217 strains of alfalfa and sweetclover bacteria were isolated from nodules growing on these plants within a restricted area. When these strains were tested on alfalfa plants, 27 percent fixed a high amount of nitrogen, 21 percent fixed a small amount of nitrogen, and 52 percent fixed in-between amounts of nitrogen.

Data are available that show the more effective strains of legume bacteria can increase the yield or protein content of legumes as much as 20 percent on the average over the natural legume bacteria in the soil.

The agricultural census figures for 1949 give the total land available for crops in the United States as more than 509,000,000 acres. Estimates show that on approximately one-fifth of this area, or about 100,000,000 acres, some legume crop—for hay, silage, seed, winter cover, or pasture—was growing. A recent grass and legume seed survey for the different States indicates that a little more than 75,000,000 acres are planted each year with some legume crop. There is no way of telling exactly what percentage of these legume seeds are inoculated with commercial cultures containing highly effective strains of legume bacteria. If an estimate is based on the total number of cultures sold each year to farmers, probably less than 20 percent, or about 15,000,000 acres, are being inoculated.

On the remaining 60,000,000 acres thousands of acres contain strains of legume bacteria capable of thoroughly inoculating the legumes planted in these soils. Farmers who have been growing legumes in such soils for long periods generally know when it is not necessary to inoculate legume seeds. On the other hand, there are many farmers who are not sure whether it pays to inoculate or not, and certainly there is every reason to believe that in many sections of the country there are many acres of land that need effective strains of legume bacteria to bring about the maximum benefits from legume crops. The facts offer a challenge to legume growers. The gains possible from the more productive strains of legume bacteria should persuade nearly every farmer to make a practical test in his own field. Only thus can he make sure that his soil does, in fact, have good inoculating power. The legumes are of great importance as crops, and a farmer can well afford to make an effort to get full value from all his legumes.

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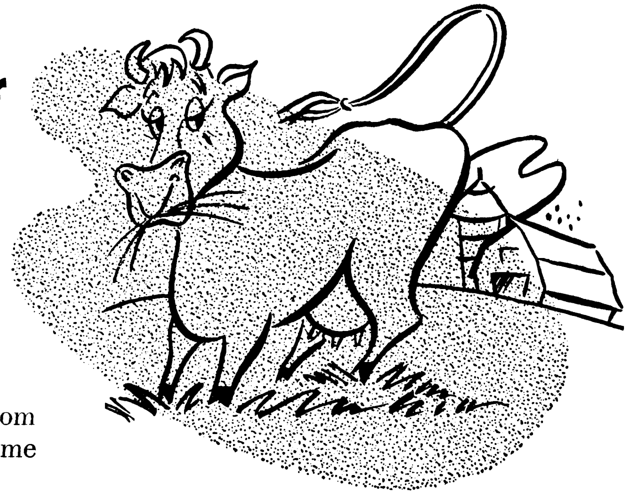
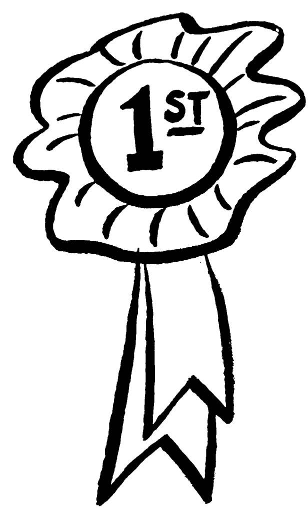


- Cut your feed costs.
- Get more income from your grass and legume acreage.
- Build up and conserve your soil.
- Save work and cut your labor bill.
- Get higher yields from other crops in your rotation.



To Improve Your Grasslands . . .

- Seed varieties and strains that are adapted to your farm and to your feeding plans. Use high-quality seed. Buy certified seed if possible.
- Sow at the right time on a properly prepared seedbed.
- Fertilize and lime your soil.
- Control weeds, brush, and insect pests.
- Practice controlled and rotation grazing, where necessary.
- Plan pastures to provide good grazing as much of the year as possible.

For Details . . . ***SEE YOUR COUNTY AGENT.***